Remarks

The claimed invention

The claimed bistable genetic toggle switch comprises two regulatory genes, R1 and R2, under control of promoters P1 and P2, wherein the expression of one regulatory gene influences the expression of the other regulatory gene, as described on p. 2, lines 15 – 19. The product of the first regulatory gene inhibits or reduces (represses) expression of the second regulatory gene. This inhibition or reduction is removed by application of a first switching agent. Similarly, the product of the second regulatory gene inhibits or reduces (represses) expression of the first regulatory gene, and this inhibition or reduction is removed by application of a second switching agent. Components of the toggle switch are selected so that the first switching agent causes the toggle switch to switch from a second stable state to a first stable state and the second switching agent causes the toggle switch to switch from a first stable state to a second stable state. Since the states are stable, one the toggle switch has entered one of the two states, it remains in that state without requiring continued presence of the switching agent that caused it to enter the state.

In other words, expression of the first regulatory gene product has a stable inhibitory effect on expression of the second regulatory gene product. This inhibitory effect can be overcome by an appropriate stimulus (switching agent) that allows expression of the second regulatory gene product. Expression of the second regulatory gene product switches off the expression of the first regulatory gene product and establishes a new stable expression state. Once established, this new expression state is stable and does not require further exposure to the switching agent (p. 3, lines 8-14). The switch does not occur in the absence of a switching agent (p. 3, lines 3-5). Similarly, expression of the second regulatory gene can be switched off, and the expression of the first regulatory gene re-activated, by applying a second switching agent that allows expression of the first regulatory gene product. Thus, the state of the claimed invention can be switched and reversed.

Rejections under 35 U.S.C. § 101

The Examiner has rejected claims 12, 15, and 16 as being directed to non-statutory subject matter. In particular, the Examiner asserts that claims 12, 15, and 16 can reasonably read to encompass a human cell in a human body, or even a human. While not conceding the correctness of the Examiner's position, claim 12 has been amended to include the term "isolated", as suggested by the Examiner, with the understanding that "isolated" shall mean that the host cell is not an integral part of a human body. Claims 15 and 16 depend from claim 12 and therefore incorporate this limitation. Claim 13 has been rewritten to depend from claim 1.

Rejections under 35 U.S.C. § 102

Claims 1-7 and 10-16 stand rejected under 35 U.S.C. § 102 as being anticipated by Bailey, et al., hereinafter "Bailey". Applicants respectfully traverse the rejection and have amended claim 1 to more clearly point out features of the claimed invention that distinguish it from Bailey. In particular, claim 1 has been amended to recite that the components of the claimed toggle switch are selected so that the toggle switch is capable of existing in either of two stable states in the absence of a switching agent and that the toggle switch can be switched between the two states by application of appropriate switching agents, as described throughout the specification, e.g., at p.2, lines 10-14. As described further below, Applicants submit that the constructs described by Bailey lack these properties.

Bailey teaches dual operon constructs that comprise two repressors, R and R2, under control of two promoters, wherein R2 controls (represses) transcription of the structural gene that encodes R, and R controls (represses) transcription of the structural gene that encodes R2 (col. 6, lines 19-28). The operon that encodes R2 also contains a sequence that encodes a product of interest. The constructs of Bailey allow one to maintain cells in a state in which transcription from the first operon, which includes both the structural gene encoding R2 and the sequence that encodes a product of interest is repressed by a repressor (R) transcribed from the second operon. Bailey further teaches that suppression of expression by a repressor can be reversed by reducing the concentration of the repressor or by neutralizing the repressor with an inducer (col. 4, line

67 – col. 5, line 3). According to Bailey's teachings, in order to achieve expression of R2 and production of the protein, one either reduces the concentration of R or applies an inducer that "neutralizes" R. It is noted that Bailey does not in fact teach how any methods by which the concentration of the repressor can be reduced.

The constructs of Bailey can thus exist in an initial state in which R is produced and represses the promoter that controls transcription of the sequence that encodes R2 and the sequence that encodes the protein of interest. In the absence of an inducer or hypothetical agent that reduces expression of R, this state is presumably stable. Application of an inducer reduces the functional activity of R, thereby allowing transcription from the promoter that controls transcription of the sequence that encodes R2 and the sequence that encodes the protein of interest. The construct thus enters a second state in which R2 and the protein of interest are produced.

Unlike the instantly claimed invention, there is no indication in Bailey that the second state is stable in the sense that it is maintained after the inducer is no longer applied. In his discussion of mathematical models for induced conditions, under which the protein of interest is expressed, Bailey assumes that the inducer that inhibits R (IPTG) is continuously present at a concentration of 10^{-3} M (col. 8, lines 41-43). In his description of an actual embodiment of his system, he adds IPTG and shows induction of CAT expression (col. 12). However, he provides no evidence to suggest that CAT expression would continue upon removal or degradation of the inducer, and there is no reason to believe that this would be the case. Rather, Applicants submit that removal or degradation of the inducer would result in decreased inhibition of repressor R and/or would permit activity of any newly synthesized R (which would occur due to leakiness of the promoter that controls expression of R unless minimized by selection of an appropriately non-leaky promoter, as taught in the instant invention). Decreased inhibition of R and/or activity of newly synthesized R would then reduce expression of R2 and allow a further increase in expression of R. Thus once the inducer is no longer present, Applicants submit that the system of Bailey does not remain in a stable state in which R2 and the protein of interest are stably expressed, but rather returns to its original state in which R is stably expressed.

The claimed invention resembles that of Bailey in that both include first and

second promoters that can be repressed by first and second repressors, wherein expression of the first repressor is under control of the second promoter and expression of the second repressor is under control of the first promoter. However, merely providing a system in which one operon encodes a repressor that regulates transcription from the other operon, and the other operon encodes a repressor that regulates transcription from the first operon, as taught by Bailey, is not sufficient to achieve the properties of Applicants' invention as recited in the instant claims. That this is the case is confirmed by the fact that a construct, pIKE105, having the configuration described above, did not exhibit bistability (p. 44, lines 15-23). Instead, the various components of the system must be selected in accordance with the teachings of the instant application in order to achieve the features that distinguish the claimed invention from that of Bailey, e.g., the ability to switch from the first state to the second state and from the second state to the first state in the presence of appropriate switching agents, and the ability to remain stably in either of the two states even in the absence of a switching agent. In accordance with the teachings of the instant application, properties such as promoter strength, repressor strength, sequence of the ribosome binding sites, etc. must be appropriately selected so that bistability can be achieved.

In particular, Applicants teach that the regulatory gene products should be balanced such that each regulatory gene product has a similar inhibitory effect on expression of the other regulatory gene product (p. 3, lines 15-17) The inhibitory strengths can be balanced by appropriate selection or modification of expression levels and functional activity. For example, a first regulatory gene product with high functional activity can be balanced with a second regulatory gene product that has a lower functional activity and is expressed at a higher level (p. 7, line 28 – p. 8, line 3). In addition, Applicants teach that in order to prevent switching in the absence of a switching agent (i.e., to ensure stability), the inhibitory properties of each gene must be sufficiently strong to prevent expression of the other gene from establishing itself (p. 7, lines 18-20).

Applicants provide further details regarding guidelines for selection and/or design of toggle switch components to achieve bistability, methods of modifying initially selected components, methods of testing components and testing toggle switches for bistable behavior, etc., in Example 1 (p. 20, line 21 – p. 29, line 24). For example

promoters with closely matched transcription efficiencies are preferably selected (p. 21, lines 4-5). Non-leaky repressors are preferably selected (p. 21, lines 10-16). In Example 2 (p. 30, line 23 – p. 36, line 22), Applicants present a mathematical analysis of bistability that can be used to select toggle switch components and determine whether a toggle switch constructed using these components will exhibit bistable behavior. Using the guidelines provided in Example 2, it is possible to determine which combinations of components will result in bistable behavior and which will not.

In Example 3 (p. 36, line 24 – p. 47, line 25) , Applicants present details regarding construction of a number of bistable genetic toggle switches with components (e.g., promoters, repressors, ribosome binding sites, etc.) selected in accordance with the invention. It is noted that the bistable toggle switches described in Example 3 differ significantly from those of Bailey in order to allow switching between two stable states. For example, the pTAK constructs contain a first promoter/repressor pair consisting of the P_{trc}-2 promoter and the lac repressor (lacI), and a second promoter/repressor pair consisting of the P_Ls1con promoter and a temperature sensitive mutant of the λ repressor (cIts). Thus switching can be achieved by a pulse of IPTG or a thermal pulse (p. 37, lines 12-23). It is noted that the a switching agent can be a natural or synthetic molecule such as a protein or nucleic acid, peptide nucleic acid or small organic or inorganic molecule, or a physical property, for example, temperature, light, osmotic pressure, pH, or membrane potential (p. 11, lines 5-9). The P_Ls1con promoter differs from the wild type P_L promoter in that it is engineered to be weaker than the extremely strong wild type PL promoter, in order to achieve better balance with the P_{trc}-2 promoter (p. 39, lines 8-12) The pIKE plasmids contain a first promoter/repressor pair consisting of the PLtetO-1 promoter and the TetR repressor. Switching can be achieved by a pulse of IPTG or a pulse of anhydrotetracycline (p. 37, lines 12-23).

In contrast, the constructs of Bailey contain a contain a first promoter/repressor pair consisting of the tac promoter (which is similar to P_{trc}-2) and the lac repressor, and a second promoter/repressor pair consisting of the wild type P_L promoter and a wild type λ repressor (cI) (col. 9, lines 18-19). Thus the constructs of Bailey do not exhibit features such as balanced promoter efficiencies and repressor activities that allow bistability. In addition, the promoter/repressor pair consisting of the P_L promoter and a wild type λ

repressor (cI) is not switchable by a switching agent. Thus the constructs of Bailey do not exhibit bistable behavior and are not switchable between two stable states by application of first and second switching agents, as in the case of the claimed bistable genetic toggle switches. Applicants therefore submit that Bailey does not anticipate the instantly claimed invention. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 8 and 9 stand rejected under 35 U.S.C. § 103 as being unpatentable over Bailey, et al. The Examiner applies Bailey as in the rejections under 35 U.S.C. § 102 and asserts that it would have been obvious to modify the systems taught by Bailey to include a second sequence of interest operatively linked to the operon that does not already contain a sequence of interest. Applicants submit that, as described above, Bailey does not anticipate the toggle switch of claim1. Therefore, even if motivation to modify Bailey to include a second sequence of interest operatively linked to the operon that does not already comprise a sequence of interest existed, and even if there was a reasonable that doing so would successfully allow one to regulate expression of more than one gene product and obtain the second gene product in a controlled manner, as asserted by the Examiner, Applicants submit that resulting system would not meet the limitations of claims 8 and 9. In particular, the resulting system would not be capable of being stable in a first state or in a second state in the absence of a switching agent and of switching from the first stable state to the second stable state and from the second stable state to the first stable state upon application of appropriate switching agents, as required in claim 1, from which claims 8 and 9 depend. As described in MPEP §706.02(j) and in *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991), one of the requirements to establish a *prima facie* case of obviousness is that the prior art reference (or references when combined) must teach or suggest all the claim limitations. Applicants submit that this requirement has not been met. Withdrawal of the rejection is respectfully requested.

Double Patenting

Claims 1-16 stand provisionally rejected as being unpatentable over claims 1-15 of copending Application No. 09/872,339. The Examiner states that in the invention

claimed in the '339 application, the first and second promoters are active in the absence of a repressor but only the first promoter is necessarily inducible by the addition of a switching agent. The Examiner further states that the second promoter, while regulated by a repressor, is not necessarily derepressed in response to a switching agent. The Examiner then asserts that since the claims of both applications are directed to dual expression constructs, it would have been obvious to practice the invention claimed in the '339 application using promoter/operator pairs both of which are responsive to switching agents. Applicant respectfully disagrees with these assertions, particularly as applied to the claims in both applications as currently amended to more clearly point out the different features of the claimed genetic switches, for each of the following reasons.

Firstly, the Examiner is incorrect in stating that the first and second promoters are active in the absence of a repressor in the invention claimed in the '339 application. This property correctly describes the promoters of the instant claims. However, in preferred embodiments of the invention claimed in the '339 application, the inducible promoter is substantially inactive in the absence of a threshold concentration of an activating agent, i.e., the default state of the inducible promoter (i.e., the state in the absence of the activating agent) is substantially inactive (p. 8, lines 10-16, and p. 20, lines 3-4 of the '339 application). Thus the inducible promoter of the '339 claims requires a threshold amount of the activating agent in order to be active, even in the absence of a repressor, unlike the promoters of the instant claims, which do not require an activating agent but are constitutively active in the absence of a repressor (p. 20, lines 17-22 of the '339 application).

Secondly, while the dual construct expression systems of the instant application and the '339 application both contain the same overall configuration comprising two promoters, each of which controls expression of a repressor that represses transcription from the other promoter, the promoters suitable for use as the inducible promoter of the claims in the '339 application are distinct from those suitable for use in the instantly claimed invention. The inducible promoters of the '339 claims are capable of being activated by an activating agent and of being suppressed by a repressor (p. 8, lines 14-19). In particular, the agent that activates the inducible promoter of the '339 claims does not do so by interfering with a regulatory gene product, as do the switching agents of the

instant claims (see p. 10, line 29 – p. 11, line 4, of the instant application, where the mechanism of action of the switching agents is described, and p. 7, lines 15-23 of the '339 application, which contrasts the activating agents of the '339 application with the switching agents useful in a bistable toggle switch). Furthermore, expression from the inducible promoter in the '339 claims can be turned on by a threshold concentration of the activating agent even in the presence of repressor (see, e.g., p. 6, lines 5-8, p. 20, lines 3-6, and p. 21, lines 7-13, of the '339 application). For example, the activating agent may increase expression of the gene(s) under its control by increasing transcription, RNA stability, translation, protein stability, or a combination of these (p. 11, lines 1-20, of the '339 application).

Table 1 (p. 9 of the '339 application) lists examples of inducible promoters and corresponding activating agents suitable for use as inducible promoters. These promoters are distinct from those listed in Table 1 of the instant application (p. 27), which are suitable for use in the instant claims but not as inducible promoters in the claims of the '339 application. One of ordinary skill in the art reading the '339 application would recognize that the promoters listed in Table 1 of the '339 application are inducible, as described above, and that they have properties distinct from those described in the instant application. One of ordinary skill in the art, reading the specification of the '339 application, would recognize that these properties are necessary for the adjustable threshold switch to function appropriately. Thus one of ordinary skill in the art would not have been motivated to use promoters suitable for use in the instant claims as the inducible promoter when practicing the claims of the '339 application because doing so would not result in an adjustable threshold switch with the properties required by the '339 claims. Withdrawal of the rejection is respectfully requested.

In light of the foregoing Amendment and Remarks, Applicants respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful or if questions arise regarding the amendment proposed above, please do not hesitate to contact the undersigned at (617) 248-5071.

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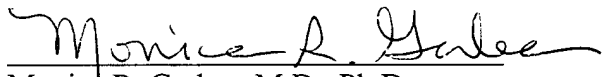
Respectfully submitted,



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